Toxicity of marine metal mixtures is concentration- and metal combination- dependent

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In polluted areas (e.g. harbours) mussels are frequently exposed to a mixture of different metals. According to available literature, mussel larvae are rather insensitive to metals such as zinc, nickel and cadmium with reported no observed effect concentrations (NOEC) that are much higher than the environmental concentrations. However, mussel larvae are very sensitive to Cu, resulting in possible adverse effects at observed environmental concentrations. When mussel larvae are exposed to metal mixtures the resulting toxicity might not be the sum of the individual effects. To date the outcome of this, more realistic, exposure scenario is unknown.

To assess the influence of binary mixtures on mussel larvae (*Mytilus edulis*) development, a total of 6 full factorial experiments (between 6*6 and 7*11) were performed. Each experiment was conducted according to the ASTM E 724-98 guidelines. At the end of the test the ratio of developed larvae versus deformed larvae was calculated for each mixture. Each experiment was performed at least twice to ensure reproducibility of the results. R statistics were used to analyse the results, calculate the concentration at which 50% of the larvae were deformed (EC50) and determine whether or not synergistic or antagonistic effects occur.

The individual EC50s found in this study were similar to the values reported in the literature. The influence of Zn on the Cu toxicity could be predicted well, assuming the concentration addition model although some antagonistic effects were observed at low Cu concentrations. This was not the case for the Cu/Ni mixture. On the one hand, there was a strong synergistic effect on Cu toxicity when the larvae were exposed to Ni concentrations as low as 2- 5% of the Ni EC50 (8-20 µg $Ni.L^{-1}$). On the other hand, high Ni concentrations (>200µg.L^{-1}) had an opposite (antagonistic) effect. Additionally the nickel toxicity increased considerably with increasing Cu toxicity. The dose level-dependent effect of the mixtures described above was reproducible in the different experiments.

For the first time this study shows that when mussel larvae are exposed to a binary metal mixture the toxicity of both metals can increase drastically. This is especially disturbing because not only does the already very low Cu EC50 decreases even further, the nickel EC50 plummet to a concentration that has been reported in polluted areas even though it was previously presumed harmless to mussel larvae.

More generally, the results indicate that a high tolerance to a certain chemical (e.g. Ni) does not automatically mean that this chemical is harmless. This study shows that it is important to test low (environmentally realistic) concentrations in mixture studies. Indeed if only a more conventional Cu/Ni experimental design would have been assessed (e.g. NOEC, EC10,...), the synergism at low Ni concentrations would have been missed.